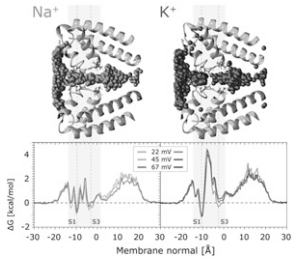
hydrated at all times, even when bound. The ion positions were correlated with electron density in selectivity filter of the crystal structure. Remarkably, and in stark contrast to K-channels, ionic conduction was found to be independent of net water flux, which was zero for all applied voltages and ionic species. This zero water transport was found to result from the balance of two large and opposing water fluxes of equal magnitude.



680-Pos Board B449

Investigating the Voltage Sensor Domains of Nav1.4, its Structural and Functional Properties via Histidine Scanning Mutagenesis

Pascal Gosselin-Badaroudine[1], Lucie Delemotte[2], Adrien Moreau[1], Michael L. Klein[2], Mohamed Chahine[1].
[1]Centre de Recherche de l'Institut Universitaire en Santé Mentale de Québec, Québec, QC, Canada, [2]Institute of Computational Molecular Science, Temple University, Philadelphia, PA, USA.

Mammalian sodium channels are composed of four homologous domains (DI, DII, DIII and DIV). Each domain is composed of six helical transmembrane segments (S1-S6). Together, the folding of S5-S6 segments of all the domains form the pore domain (PD). The S1-S4 segments of each domain form the voltage sensor domain (VSD). Recently, mutations in the VSD of Nav channels have been linked to pathologies such as hypo- and normokalemic periodic paralysis and recently dilated cardiomyopathyGosselin-Badaroudine P., et al.,(2012): PlosONE, 7(5):e38331.

Here we use the histidine scanning mutagenesis technique to investigate the role of the positively charged amino acids along the S4 segments of each domain. Detecting a proton current at hyperpolarized potentials indicates that the mutated residue is located in the gating charge transfer center of the protein. Also, proton transport implicates that the mutated residue moves across the gating charge transfer center during activation. Furthermore, a shift in the Q-V curve indicate that the mutated residue plays an important role in the stabilization of the S4 segment in its activated or resting position.

The results lead to the creation of the first structural model of the VSDs of a mammalian sodium channel in its resting state. This structural model features hydrophobic septa of different dimensions. Indeed, the VSD of the fourth domain displays a hydrophobic septum much larger than the septa of the other domains. This difference of the VSD of the forth domain provides a rationale for its late onset in the activation sequence and the fact that no gating pore current have been uncovered in the VSD of DIV of mammalian sodium channels.

681-Pos Board B450

Atrial Selectivity in Sodium Channel Block by Amiodarone

Tomoyuki Suzuki, Mikio Morishima, Sara Kato, Yoshio Takemoto, Hiroki Takanari, Norihiro Ueda, Yukiomi Tsuji, Haruo Honjo, Kaichiro Kamiya.
Research Institute of Environmental Medicine Nagoya University, Nagoya, Japan.

Introduction: Na^+ channel blockers are usually applied to atrial fibrillation (AF), but may sometimes cause cardiac contractile dysfunction. However, amiodarone, a multi-channel blocker with Na^+ channel block, does not induce cardiac dysfunction. In this study we tested the hypothesis that Na^+ channel block by amiodarone is selective in atrial myocytes (AM) compared to ventricular myocytes (VM). **Methods and Results:** Na^+ currents (I_{Na}) and resting membrane potentials (RMPs) were measured using whole cell patch-clamp technique in isolated rabbit AM and VM. Amiodarone inhibited I_{Na} in AM (IC_{50}: 1.4 ± 0.3μM; n=8) much more than in VM (40.4 ± 11.9μM; n=7; $P<0.01$). Amiodarone at 10μM dramatically shifted steady state inactivation curve to hyperpolarized direction in AM (−19.6 ± 2.1mV shift; n=12) compared to VM (−6.3 ± 0.8mV shift; n=13; $P<0.01$). In mexiletine, there was no significant difference in I_{Na} inhibition between AM and VM. The shifts of inactivation curves by mexiletine at 10μM were comparable in AM and VM. RMPs in AM (−75.0 ± 1.3mV; n=4) were more depolarized than in VM (−82.1 ± 1.1mV; n=9; $P<0.01$). In the absence of drugs, the half inactivation voltage in AM ($V_{1/2}$: −89.7 ± 0.9mV; n=19) was 12.5 mV more negative than VM (−77.2 ± 0.6mV; n=20; $P<0.01$). Furthermore, we evaluated the effects of amiodarone and mexiletine on conduction velocity (CV) in Langendorff-perfused rabbit hearts by optical mapping system. The decrease of CV by amiodarone at 5μM was significantly larger in atrium (−34.3 ± 5.6%; n=5) compared to ventricle (−4.8 ± 1.0%; n=5; $P<0.01$). However, the reduction of CV by mexiletine at 5uM in atrium was smaller than in ventricle. ***Conclusion:*** Amiodarone preferentially inhibits I_{Na} of AM compared to VM. This atrial-selective Na^+ channel block by amiodarone may contribute to treating AF without affecting ventricular contractility.

682-Pos Board B451

Wild-Type Sodium Channels and 'Atypical' Brugada Syndrome Mutants Interact through C-Terminal Region

Malcolm Hoshi.
Case Western Reserve University, Cleveland, OH, USA.

Introduction: Brugada syndrome (BrS) is an inherited cardiac disorder that can be caused by mutations in the cardiac sodium channel gene resulting in a reduction of sodium currents. However, several 'atypical' sodium channel mutations identified in patients show miminal biophysical defects questioning how such mutants may produce a BrS phenotype. We have previously demonstrated that when ''atypical'' BrS mutants were co-expressed with wild-type channels (WT) mimicking the heterozygous patient genotype, this lead to a dramatic reduction in sodium currents thereby explaining the disease phenotype. Here we hypothesized that a direct interaction between atypical BrS mutants and WT is mediated via the calmodulin-binding IQ domain of the channel protein. Methods: 'Atypical' BrS mutations with minimal biophysical defects were co-expressed with either WT or WT channels containing a mutated calmodulin binding IQ motif (IQ/AA) in HEK293 cells. BrS mutations from all intracellular loops were studied. Biophysical properties of mutants were investigated by patch clamp. Co-immunoprecipitation and cell surface biotinylation were performed to assess interaction and channel location. Results: BrS mutants co-expressed with IQ/AA had current amplitudes restored to control levels. This abrogated any loss-of-current phenotype we observed when the ''atypical'' mutants were co-expressed with WT. Cell surface biotinylation showed a significant reduction of both WT and mutant channels at the plasma membrane on co-expression, while surface expression was restored for both channels when mutants were co-expressed with the IQ/AA construct. Importantly, while co-immunoprecipitation experiments demonstrated interactions between WT and ''atypical'' mutant channels, IQ/AA failed to interact with atypical BrS mutants. Conclusions: Our data suggest that 'atypical' BrS mutations suppress both mutant and WT channels expression at the cell surface via interactions mediated by the calmodulin-binding (IQ) domain of the cardiac sodium channel.

683-Pos Board B452

The β_1-Subunit of $Na_V1.5$ Cardiac Sodium Channel is required for a Dominant Negative Effect through α-α Interaction

Aurelie Mercier[1], Romain Clément[1], Thomas Harnois[1], Nicolas Bourmeyster[1], Jean-François Faivres[1], Ian Findlay[1], Mohamed Chahine[2], Patrick Bois[1], Aurelien Chatelier[1].
[1]Institut de Physiologie et de Biologie Cellulaire, poitiers, France, [2]Institut Universitaire en santé mentale de Québec, Quebec, QC, Canada.

Brugada syndrome (BrS) is an inherited autosomal dominant cardiac channelopathy. Several mutations on the cardiac sodium channel $Na_v1.5$ which are responsible for BrS lead to misfolded proteins that do not traffic properly to the plasma membrane. In order to mimic patient heterozygosity, a trafficking defective mutant, R1432G was co-expressed with Wild Type (WT) $Na_v1.5$ channels in HEK293T cells. This mutant significantly decreased the membrane Na current density when it was co-transfected with the WT channel. This dominant negative effect did not result in altered biophysical properties of $Na_v1.5$ channels. Luminometric experiments revealed that the expression of mutant proteins induced a significant reduction in membrane expression of WT channels. Interestingly, we have found that the auxiliary Na channel β_1-subunit was essential for this dominant negative effect. Indeed, the absence of the β_1-subunit prevented the decrease in WT sodium current density and surface proteins associated with the dominant negative effect. Coimmunoprecipitation experiments demonstrated a physical interaction between Na channel α-subunits. This interaction occurred only when the β_1-subunit was present. Our findings reveal a new role for β_1-subunits in cardiac voltage-gated sodium channels by promoting α-α subunit interaction which can lead to a dominant negative effect when one of the α-subunits shows a trafficking defective mutation.

684-Pos Board B453

Proton Modulation of Ranolazine Effects on Slow Inactivation in Sodium Channels

Colin H. Peters[1], Stanislav Sokolov[1], Sridharan Rajamani[2], Peter C. Ruben[1].
[1]Simon Fraser University, Burnaby, BC, Canada, [2]Gilead Sciences, Inc., Foster City, CA, USA.

Ranolazine is a clinically approved anti-anginal drug with potential antiarrhythmic, antiepileptic, and analgesic applications. The therapeutic effects of ranolazine are dependent on its ability to preferentially inhibit persistent currents in a variety of voltage-gated sodium channels. Extracellular acidosis, as